Chemosensory Deprivation in Juvenile Coho Salmon Exposed to Dissolved Copper under Varying Water Chemistry Conditions

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Dissolved copper is an important nonpoint source pollutant in aquatic ecosystems worldwide. Copper is neurotoxic to fish and is specifically known to interfere with the normal function of the peripheral olfactory nervous system. However, the influence of water chemistry on the bioavailability and toxicity of copper to olfactory sensory neurons is not well understood. Here we used electrophysiological recordings from the olfactory epithelium of juvenile coho salmon (*Oncorhynchus kisutch*) to investigate the impacts of copper in freshwaters with different chemical properties. In low ionic strength artificial fresh water, a short-term (30 min) exposure to 20 μg/L dissolved copper reduced the olfactory response to a natural odorant (10^{-5} M L-serine) by 82%. Increasing water hardness (0.2–1.6 mM Ca) or alkalinity (0.2–3.2 mM HCO_3^-) only slightly diminished the inhibitory effects of copper. Moreover, the loss of olfactory function was not affected by a change in pH from 8.6 to 7.6. By contrast, olfactory capacity was partially restored by increasing dissolved organic carbon (DOC; 0.1–6.0 mg/L). Given the range of natural water quality conditions in the western United States, water hardness and alkalinity are unlikely to protect threatened or endangered salmon from the sensory neurotoxicity of copper. However, the olfactory toxicity of copper may be partially reduced in surface waters that have a high DOC content.

Introduction

The neurotoxicity of dissolved copper to the sensory systems of fish has been the focus of considerable research over the past few decades. The olfactory epithelium of fish, which contains ciliated olfactory sensory neurons (OSNs) embedded in a layer of mucous, is in direct contact with surface waters and is therefore particularly vulnerable to the toxic effects of copper and other pollutants. Numerous studies have shown that copper diminishes the sensitivity and responsiveness of OSNs to chemical cues (*1*). Moreover, low-level copper exposures interfere with predator avoidance behaviors that are important for survival (*2–5*). In salmon, the relationship between a loss of sensory capacity (as measured using electrophysiological recordings) and behavioral impairment is highly correlated (0–20 μg/L Cu; (*3*)). At higher exposure concentrations (≥20 μg/L) copper causes the degeneration of the sensory epithelium (*6, 7*). These effects manifest on a time scale of hours (*3, 6*) or even minutes (*8*). This makes the fish olfactory system a particularly suitable end point for evaluating the toxicological effects of copper in the aquatic environment.

Copper is classically known to disrupt osmoregulation in fishes by interfering with sodium uptake in the gill (*9, 10*). This mechanism causes acute lethality at high exposure concentrations. The toxicity of copper to the fish gill epithelium is influenced by the hardness, alkalinity, pH, and dissolved organic matter (DOM) content of water. These constituents can protect against the acute toxicity of free ionic copper by either competing for binding sites at the sodium transporter (cations) or by reducing the bioavailability of copper via complexation (anions and DOM) (Figure S1 in the Supporting Information). The biotic ligand model (BLM) is widely used to predict the degree to which these water chemistry parameters will protect against acute lethality caused by copper binding to the fish gill (*11–13*). The BLM also provides a conceptual basis for the U.S. Environmental Protection Agency's (EPA) approach to risk assessment for metals (*14*), and is being incorporated directly into the national copper aquatic life criterion (*15*).

The extent to which the BLM can be used to estimate the sublethal neurobehavioral toxicity of copper in fish is less clear. The gill epithelium and olfactory epithelium are very different in terms of both architecture and physiological function (Figure S1). In contrast to the cellular processes underlying osmoregulation and gas exchange, the primary function of OSNs is to transduce odorant binding and receptor activation to electrical signals (action potentials) that are ultimately propagated to the olfactory forebrain. Olfactory signal transduction involves a complicated and finely tuned biochemical cascade (Figure S1) (*16*). The "ligand" for copper in the fish nose has not been identified, but may involve one or more proteins that are unrelated to sodium exchange (*17*). Accordingly, the predictive capacity of the BLM, as originally developed for gill-mediated lethality, may or may not extend to sublethal effects on the fish olfactory system.

To explore the effect of water chemistry on olfactory neurotoxicity, we used a well-established neurophysiological approach (*18, 19*) to monitor the olfactory neurotoxicity of copper in juvenile coho salmon (*Oncorhynchus kisutch*), while varying water hardness, alkalinity, pH, and dissolved organic carbon (DOC). We exposed fish to copper for 30 min at 20 μg/L (0.315 μM), a concentration that is (1) environmentally relevant for salmon exposed to copper in urban runoff (*20*) and (2) likely to produce a robust olfactory inhibition as a baseline for assessing the potential protective effects of different water chemistries (*18, 19*). To emulate the natural diversity of water chemistry conditions in Pacific salmon habitats, we adjusted each parameter to encompass the range of values reported by the U.S. Geological Survey's National Water Quality Assessment Program for freshwater streams and rivers in the Puget Sound region as well as the Willamette, Yakima, and Sacramento River drainage basins. These surface waters provide spawning and rearing habitat for salmon populations that are listed as either threatened or endangered under the U.S. Endangered Species Act (ESA; see NOAA Office

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TABLE 1. Water Quality Parameters for Artificial Freshwaters, Deionized Water (DI), Dechlorinated City Water (DCW), and Hatchery Water (HW), Including pH and Concentrations of Major Inorganic Ions, Alkalinity, Hardness, Dissolved Organic Carbon (DOC), Dissolved Copper (DCu), and Ionic Copper (ICu) [a]

	nominal			measured								alkalinity	hardness			modeled	
	Ca mM	HCO_3^- mM	DOM mg/L	pH	Ca mM	Mg mM	Na mM	K mM	Cl mM	SO_4^{2-} mM	HCO_3^- mM	mg/L as $CaCO_3$ [b]	mg/L as $CaCO_3$	DOC mg/L	DCu µg/L	ICu µg/L [c]	ICu % of DCu
water					0.001 [d]	0.002	0.002	0.003	0.028	0.01	n.a. [e]	1	1	0.25	0.04		
low-ion	0.2	0.2	0	7.5	0.203	0.097	0.188	0.02	0.413	0.09	0.25	13	30	0.1	14.05	5.08	36.2
Ca.1	0.4	0.2	0	7.5	0.432	0.099	0.199	0.021	0.845	0.09	0.25	12	53	n.m.	13.20	4.88	37
Ca.2	0.8	0.2	0	7.5	0.898	0.091	0.183	0.019	1.634	0.09	0.23	11	99	n.m.	13.03	4.98	38.2
Ca.3	1.6	0.2	0	7.6	1.820	0.093	0.183	0.021	3.380	0.09	0.25	13	190	n.m.	14.97	5.22	34.9
Alk.1	0.2	0.8	0	8.2	0.211	0.090	0.783	0.013	0.394	0.09	0.82	41	30	n.m.	16.15	0.64	4
Alk.2	0.2	1.6	0	8.5	0.197	0.091	1.551	0.014	0.432	0.09	1.57	79	29	n.m.	14.90	0.17	1.1
Alk.3	0.2	3.2	0	8.6	0.200	0.094	3.087	0.015	0.394	0.09	3.20	160	30	n.m.	15.40	0.07	0.5
Alk.3 [f]	0.2	3.2	0	7.6	0.195	0.088	3.058	0.014	0.789	0.09	2.74	137	29	n.m.	16.33	0.82	5
FA.1	0.2	0.2	2.5	7.4	0.183	0.086	0.178	0.02	0.423	0.11	0.21	11	27	1.94	17.03	0.45	2.6
FA.2	0.2	0.2	5	7.2	0.181	0.086	0.181	0.02	0.423	0.11	0.20	10	27	2.76	17.70	0.17	1
FA.3	0.2	0.2	10	7.1	0.194	0.09	0.187	0.021	0.423	0.12	0.18	9	28	6.03	18.50	0.05	0.3
NOM	0.2	0.2	10	7.2	0.181	0.086	0.181	0.02	0.423	0.11	0.20	10	27	4.11	18.30	0.16	0.9
DI [g]	n.a.	n.a.	n.a.	n.m [h]	<	<<<<<					<	<	<	0	<<	0 5	n.a.
DCW	n.a.	n.a.	n.a.	n.m.	0.400	0.078	0.13	0.01	0.194	0.03	0.84	42	49	n.m.	0.20	n.m.	n.a.
HW	n.a.	n.a.	n.a.	n.m.	1.022	0.11	6.957	0.116	1.746	0.23	3.00	150	110	4.27	4.06	n.m.	n.a.

[a] For measured parameters, values are the mean of three replicate samples. The measured concentrations of conventional cations were 93 (10% (mean (SD) of nominal values. Dissolved organic carbon (DOC) concentrations were 0.11 (0.01 mg/L in AFW in the absence of added DOM and 6.03 (0.13 mg/L in AFW amended with 10 mg/L FA. The DOC content of AFW containing 10 mg/L NOM was lower than that of AFW containing FA (4.11 (0.23 mg/L DOC). Measured concentrations of dissolved copper were 80 (9% (mean (SD) of nominal values. [b] All alkalinity present as bicarbonate. [c] Ionic copper concentrations from VMINTEQ modeling at test temperature (13 °C). [d] Reporting limit. [e] Calculated from alkalinity. [f] pH lowered with HCl. [g] All water quality parameters in DI were below reporting limits. [h] Not measured.

of Protected Resources, http://www.nmfs.noaa.gov/pr/species/Þsh/ for current species listings). This approach allowed us to assess the extent to which the neurobehavioral toxicity of dissolved copper to ESA-listed salmonids may be altered in river systems with different water chemistries.

Materials and Methods

Animals. Juvenile coho salmon (*n*) 80; 188 (2 mm, 71 (2 g [mean (SEM]) were reared at the Northwest Fisheries Science Center (Seattle, WA) and maintained under ambient lighting in dechlorinated municipal city water adjusted for hardness and pH (DCW; Table 1). Prior to copper exposures, Þsh were acclimated overnight (14–18 h) in 30 L glass aquaria containing 20 L of the appropriate artiÞcial fresh water (AFW; see below). Acclimation tanks were aerated and maintained under static conditions at 13 °C with ambient light.

Artificial Fresh Water. We used data from the U.S. Geological Survey's National Water Information System Web Site (NWISWeb; http://nwis.waterdata.usgs.gov/nwis) to identify a natural range of water chemistries in freshwater salmon habitats throughout the western United States. This included more than a decade of surface water quality monitoring (1990–2003) for streams in the Puget Sound region as well as the Willamette, Yakima, and Sacramento River basins. Monitoring data were drawn from 7–24 sites (5–21 streams) for each of the four basins. The nominal compositions of the different artiÞcial fresh waters (AFW) are shown in Table 1.

To establish a baseline toxicological response, juvenile coho were exposed to copper in a low ionic strength AFW (0.2 mM Ca, 0.2 mM Na, 0.1 mM Mg, 0.01 mM K; Table 1). Hardness was increased by adding calcium as $CaCl_2$ (to 0.4, 0.8, 1.6 mM Ca). Although in natural waters hardness is inßuenced by cations other than calcium (e.g., Mg^{2+}), the term "hardness" is used interchangeably with calcium concentration here. Alkalinity was increased by adding bicarbonate as $NaHCO_3$ (to 0.8, 1.6, 3.2 mM HCO_3^-). In the highest alkalinity water, pH was reduced from 8.6 to 7.6 with 0.37 N HCl. Reference fulvic acid (FA) and natural organic matter (NOM) isolated from the Suwannee River, Georgia were obtained from the International Humic Substances Society (IHSS; St. Paul, MN, http://www.ihss.gatech.edu) and were added to AFW to increase DOM (to 2.5, 5, 10 mg/L FA, or 10 mg/L NOM).

For dissolved copper exposures, AFW was made daily from ion stock solutions using salts ($CaCl_2 \cdot 2H_2O$, $NaHCO_3$, $MgSO_4 \cdot 7H_2O$, KCl) from Sigma-Aldrich (www.sigmaaldrich.com). The exception was AFW containing DOM, which was prepared 24 h prior to use to allow the amended copper and DOC to equilibrate (*21*). Copper was added to AFW from a stock solution of 1 g/L Cu (2.68 g/L $CuCl_2 \cdot 2H_2O$) to reach a nominal concentration of 20 µg/L (0.315 µM). All stock solutions were prepared weekly and stored at 5 °C. Deionized water (DI) was used in all preparations. Measured values for each ionic constituent, alkalinity, pH, hardness, DOC, dissolved copper, and ionic copper are shown in Table 1.

Electrophysiological Recordings. Odor-evoked Þeld potential recordings were used to measure the relative impacts of dissolved copper in different AFWs. The experimental procedures for recording electro-olfactograms (EOGs) have been published previously (*19*); see also (*3, 8, 22*). Trials began by perfusing the olfactory chamber with AFW for 15 min. Over the subsequent 15 min, precopper exposure EOGs were obtained by delivering a natural odorant (10^{-5} M L-serine) to the sensory epithelium for 10 s every 5 min. Following 30 min of continuous AFW perfusion, the olfactory chamber was perfused with AFW containing 20 µg/L Cu for 30 min. The periodic presentation of odor pulses continued during the exposure interval. The maximum negative phasic displacement from the electrical baseline (the odor-evoked EOG) was used to quantify the olfactory response (in mV). For each individual Þsh, the amplitude of the postexposure EOG was divided by the pre-exposure amplitude to calculate a relative olfactory response (Figure S2). EOG recordings were collected from *n*) 5–10 Þsh for each AFW except for AFW containing NOM (*n*) 4).

Chemical Analyses. Measurements of conventional ions and dissolved copper were conducted on triplicate samples

of AFW collected from the experimental exposure system (the tube delivering solutions to the salmon olfactory chamber). The DI water used to make AFW was also analyzed as were the waters used to rear and maintain the fish (hatchery water) and the supply of dechlorinated city water (DCW) to the hatchery. Analyses for conventional ion composition, hardness, and alkalinity were conducted by an EPA-certified laboratory (AmTest Inc., Redmond, WA) using standard methods. Samples containing dissolved copper were analyzed by an EPA-certified laboratory (Frontier Geosciences Inc., Seattle, WA) using inductively coupled plasma mass spectrometry (ICP-MS) with a detection limit of 0.04 μg/L (see ref (*22*)).

For a subset of AFWs (low-ion control, 0 FA, 2.5 FA, 5 FA, 10 FA, 10 NOM, HW), dissolved organic carbon samples were analyzed by Shimadzu TOC-Vcsh (University of Washington Oceanography Technical Services; Seattle, WA). A Fisher Scientific Accumet AB15 (calibrated daily) was used to measure the pH of AFW samples.

Copper Ion Measurement. Free ionic copper concentration [Cu^{2+}] was measured at room temperature (22 °C) on AFW collected from the olfactory perfusion system with a cupric ion selective electrode (Cu ISE) (Orion 94-29; Thermo Orion Inc., Beverly, MA) paired with a double junction reference electrode (Orion 90-02) and an ISE meter (Orion 720A). The copper ISE electrode was maintained and calibrated daily for low level [Cu^{2+}] measurements according to manufacturer's specifications (*23*). For measurements that fell within the calibration range (low-ion and Ca manipulations), [Cu^{2+}] was determined by linear interpolation between the adjacent calibration points. For the low-copper samples that fell below the calibration range, [Cu^{2+}] was expressed in mV from the mV reading for the lowest calibration standard (0.6 μg/L) in the associated calibration curve (0.6–30.5 μg/L Cu).

Modeling Copper Speciation and Median Effect Concentrations. Methods for modeling copper speciation, the use of the Biotic Ligand Model to predict median lethal concentrations, and the use of regressions to estimate the median inhibitory concentration for olfactory inhibition are presented in the Supporting Information.

Statistical Analyses. Statistical comparisons included one-way analysis of variance (ANOVA), simple linear regression, and *t* tests. Linear regressions were used to test the effect of hardness, alkalinity, and DOC on the precopper EOG amplitude and the relative EOG amplitude at the end of the copper exposure. An ANOVA was used to identify differences in the average postexposure EOG amplitude among fish in each of the AFWs. Differences from unexposed controls were subsequently tested with Dunnett's posthoc test. *t*-Tests were used to test for differences in relative EOG amplitude for (1) 10 mg/L NOM vs FA and (2) low vs high pH for 3.2 mM HCO_3^-. The relative importance of each water parameter (hardness, alkalinity, DOC) for predicting copper toxicity at the gill vs the nose was assessed by examining the slopes for the LC50 (via gill toxicity) vs the IC50 (for olfactory toxicity). Slopes were considered not statistically different if the LC50 slope coefficient fell within the 95% confidence interval for the IC50 slope coefficient. All statistics were performed using SPSS 11.5 for Windows (www.spss.com) with a significance level of $R = 0.05$.

Results

Measured and Modeled Composition of Artificial Fresh Water. The measured concentrations of conventional cations and dissolved copper were 93 ± 10% (mean ± SD) and 80 ± 9% of nominal values, respectively (Table 1). Measured DOC concentration and nominal FA concentration were highly correlated (Pearson correlation coefficient; $r^2 = 0.990$, df = 14, $p < 0.001$; DOC = 0.58 × FA + 0.15). Measured and modeled concentrations of free copper were in close agreement and ranged from 38% of dissolved copper concentration in AFW with added calcium to <1% of dissolved copper concentration at the highest concentrations of bicarbonate or DOM (Table 1).

Influence of Water Chemistry on the Olfactory Neurotoxicity of Copper. Consistent with previous studies (*8, 22*), a short-term (30 min) exposure to dissolved copper significantly reduced the amplitude of the juvenile coho olfactory response to the natural odorant 10^{-5} M L-serine. In the control (low-ion) AFW, 20 μg/L copper reduced the size of the odor-evoked EOGs by 82% relative to pre-exposure responses (Figure 1). The relative olfactory response of noncopper exposed controls in low-ion AFW did not decline appreciably over the duration of the recording interval. The size of the olfactory response at the end of the experimental perfusion was 102 ± 6% (mean ± SE) of the initial response (Figure 1). The responsiveness of the olfactory system during the pre-exposure perfusion interval was unaffected by changes in the concentration of bicarbonate (simple linear regression: $p = 0.824$) or DOC ($p = 0.460$). The amplitude of the odor-evoked EOG showed a slight but significant negative relationship to concentration of calcium ($r = -0.315$; slope = −0.268, $p = 0.003$).

Copper exposures in each of the different AFWs significantly reduced the coho olfactory response relative to unexposed controls ($F_{11,67} = 31.658$, $p < 0.001$; Dunnett's post hoc: all $p < 0.001$). The only exception was AFW containing the highest concentration of FA (10 mg/L), in which the olfactory responses of copper-exposed fish were indistinguishable from controls (Dunnett's post hoc: $p = 0.077$). None of the other AFW formulations conferred complete protection against the neurotoxic effects of copper (Figure 1). Artificial water containing 10 mg/L FA was more protective than AFW containing 10 mg/L NOM (*t* test: $p = 0.005$), which likely reflects the higher organic carbon content of the former. The inhibitory effect of copper on odor-evoked EOGs was not influenced by pH (Figure 1; *t* test: $p = 0.939$).

Increasing water hardness, alkalinity, or DOM reduced copper toxicity (simple linear regression: $p < 0.001$), albeit by different degrees. As shown in Figure 1A and B, the influence of water hardness and alkalinity was relatively minor. On a unit-equivalent (mg/L) basis, the slope of the relationship between relative EOG amplitude and the concentration of each water chemistry constituent was more than an order of magnitude lower for calcium or bicarbonate than for DOM (Figure 1C). On a molar-equivalent basis, this relationship differed by more than 2 orders of magnitude (Table S1). Therefore, the protective effects of DOM are much greater than the protection afforded by either water hardness or alkalinity.

Water Chemistry in Western River Systems Is Unlikely to Protect Salmon from the Olfactory Toxicity of Copper. Based on previous monitoring investigations in west coast streams (U.S. Geological Survey NWISWeb; http://nwis.waterdata.usgs.gov/nwis), calcium and alkalinity are unlikely to substantially reduce the impacts of dissolved copper on salmon olfaction in natural habitats. In the surveyed streams of the Puget Sound, Willamette River, Yakima River, and Sacramento River basins, environmental calcium did not reach concentrations sufficient to reduce copper toxicity by half (2.8 mM; 113 mg/L; Figure 2, top panel). Similarly, less than 1% of surveyed surface water samples had bicarbonate concentrations that would reduce copper toxicity by half (6.5 mM; 398 mg/L). By contrast, many western river systems are likely to contain sufficient DOC to at least partially reduce the olfactory neurotoxicity of copper (Figure 2, bottom panel). For example, 19% of all surface water samples collected in the above basins contained enough DOC to reduce sublethal copper toxicity by approximately half. Only a small minority

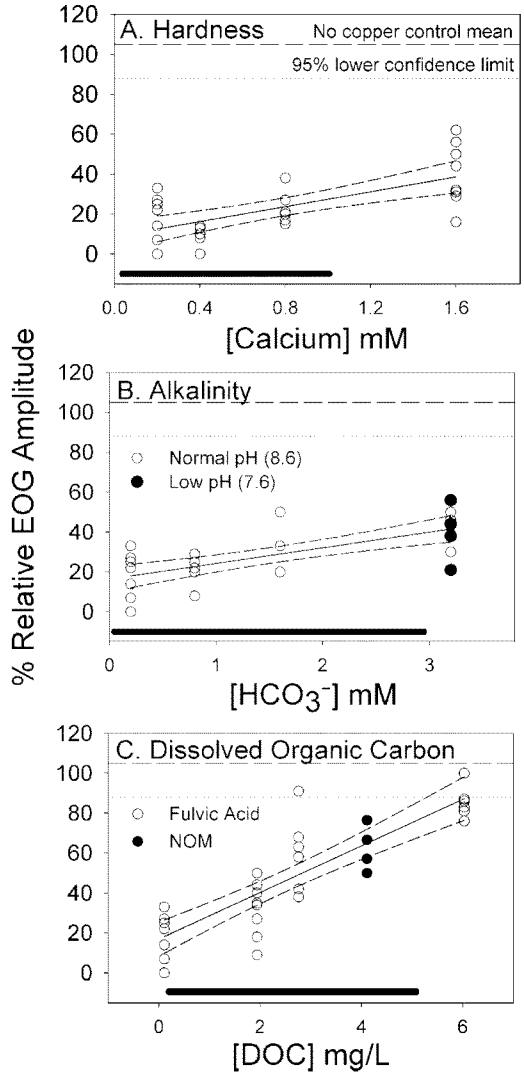

FIGURE 1. The olfactory response of juvenile coho salmon is reduced in the presence of copper. Relative olfactory response to 10^{-5} M L-serine after 30-min exposure to 20 μg/L (0.315 μM) copper in artificial fresh water of varying calcium (A), bicarbonate (B), or dissolved organic carbon (DOC) concentration (C). NOM) natural organic matter. Dashed and dotted lines across the top of each panel are, respectively, the mean and lower 95% confidence limit for the mean olfactory response of negative control fish. Each data point represents the amplitude of the olfactory response of one individual relative to its precopper response amplitude (Figure S2). Regression lines and 95% confidence intervals of the regressions are also included. Dark bars along the *x*-axes show the range of average concentrations of each water chemistry parameter surveyed in streams in the western U.S. subregions of Puget Sound, Willamette River, Yakima River, and Sacramento River (U.S. Geological Survey; NWISWeb).

of streams (6%) contained enough DOC (g6 mg/L) to eliminate copper toxicity altogether (i.e., where EOG amplitudes would be expected to reach the lower 95% conÞdence limit for unexposed controls). On a basin-speciÞc basis, stream sites containing g6 mg/L DOC represented 10% of samples in the Puget Sound basin, 2% in the Willamette basin, 7% in the Yakima basin, and 5% in the Sacramento basin.

Comparison of Copper Toxicity to the Salmon Gill and Nose under Different Water Chemistries. The Biotic Ligand Model (BLM) is widely used to estimate the acute lethality of metals under varying water chemistry conditions (*15*). We evaluated the extent to which the predictive capacity of the BLM extends to the salmon olfactory system. SpeciÞcally, for each water quality parameter (hardness, alkalinity, and DOC), we compared the slopes of the relationships of the median lethal concentration (50% mortality, or LC50, as predicted by the BLM) and the median inhibitory concentration (copper concentration causing a 50% loss of chemosensory capacity, or the IC50, as determined empirically from results presented here and in a previous study [ref (*19*)] (see Supplemental Methods in the Supporting Information). As shown in Figure 3, water chemistry has a markedly different inßuence on these two toxicological end points, with the classical end point (acute lethality) having much greater sensitivity to variation in water hardness, alkalinity, and DOC. In comparing the two tissues, the inßuence of calcium was 3-fold greater for the gill relative to the nose. The effects of bicarbonate and DOC were 80-fold and 20-fold higher for the gill, respectively. Slopes were signiÞcantly divergent for all three parameters (LC50 slopes well above the 95% CL for IC50 slopes). The BLM also predicted a substantial decrease in the LC50 (from 312 to 111 μg/L) following a 1-unit drop in pH (from 8.6 to 7.6). However, no change in olfactory toxicity was observed in association with this change in pH in the present study (i.e., in the AFW with the highest alkalinity). These results collectively indicate that the ligands for copper in the gill and the nose are likely to be distinct.

Discussion

It is now well established that the Þsh olfactory system is particularly vulnerable to the neurotoxic effects of dissolved copper in the aquatic environment. Relative to classical toxicological measures (i.e., acute lethality), olfaction has several signiÞcant advantages as an end point for assessing the risks that copper poses to Þsh, including threatened and endangered species. First, copper toxicity in the olfactory epithelium manifests on a time scale of minutes (vs a 96-h LC50). Second, copper causes a loss of sensory function at very low, environmentally realistic exposure concentrations (at or below 5 μg/L *3*, *8*). Third, olfaction underlies an important suite of life history traits in Þsh, and copper-induced anosmia is a good predictor of impairment to critical behaviors (*3*). Finally, as we have shown in this study, the toxicity of copper to the Þsh nose is only marginally inßuenced by water chemistry over the ranges that commonly occur in river drainages of the west coast of the United States. It should therefore be considerably easier to predict the site-speciÞc toxicity of copper to the olfactory system as compared to the gill.

River systems in the western United States that provide habitat for ESA-listed PaciÞc salmon and steelhead are typically soft with a low alkalinity and a low DOC content. With a few exceptions (i.e., when DOC levels exceed 6 mg/L), the site-speciÞc chemistries of these surface waters are unlikely to completely protect the salmon olfactory system from the neurotoxic effects of dissolved copper. However, it should be noted that LC50s for copper vary by as much as 4-fold in natural waters having different sources of organic matter (*24*, *25*). We used a FA derived from Suwannee River NOM, and it remains to be determined whether copper's chemosensory toxicity will vary with NOM from different river basins in the western United States. Nevertheless, our Þndings suggest that copper originating from motor vehicles, pesticide formulations, building materials, boat yards, and other sources has the potential to interfere with the chemosensory-mediated behavior of PaciÞc salmon under the majority of exposure conditions in western U.S. streams.

Copper has been shown to disrupt olfaction in a diversity of Þshes (discussed in ref (*3*)) as well as other aquatic species (*5*). Accordingly, our current Þndings are likely to extend to

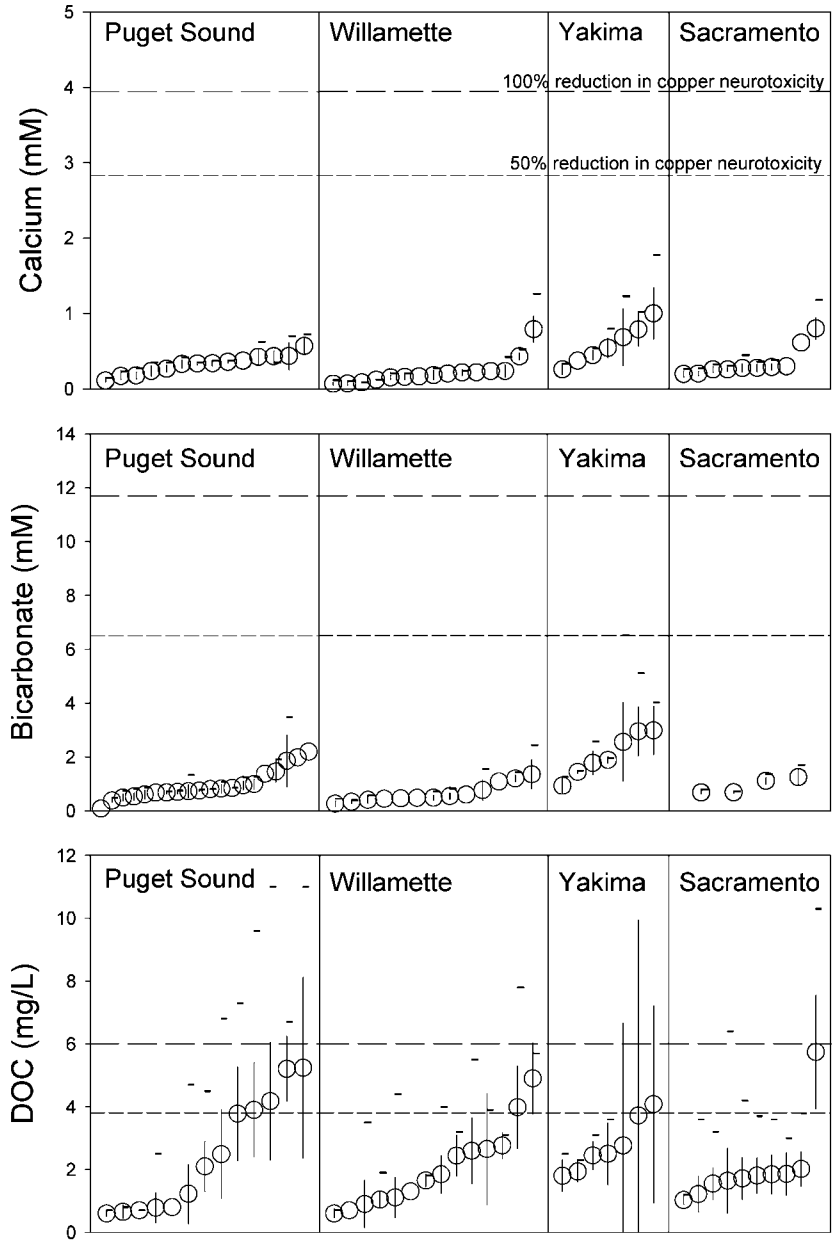

FIGURE 2. Ambient concentrations of various water quality parameters are expected to provide little or no protection against copper olfactory neurotoxicity. Concentrations of calcium, alkalinity, or dissolved organic carbon corresponding to 50% (short dashed lines) or 100% (long dashed lines) reduction in copper neurotoxicity in juvenile coho salmon exposed to 20 μg/L (0.315 μM) copper for 30 min, compared with concentrations of each parameter (ranked by average, with standard deviation, and maximum) in streams in the basins of Puget Sound, Willamette River, Yakima River, and Sacramento River (U.S. Geological Survey; NWISWeb). Reductions in neurotoxicity are based on the regression parameters in Table S1.

other Þsh species in polluted freshwater habitats worldwide. The extent to which our results can be extended to Þshes in estuaries or saltwater is less clear. This is because the cation and DOC contents of these habitats are considerably higher than the range of values examined here. Also, the salmon olfactory system undergoes physiological changes when these anadromous animals migrate from freshwater to the ocean, and this may alter the expression or function of the as-yet unidentiÞed ligand(s) for copper. Additional studies using seawater-acclimated Þsh are therefore recommended.

Copper has a higher afÞnity for bicarbonate (log K ❯ 14.3) than for the biotic ligand in the Þsh gill (log K ❯ 7.4) (*13*). At the gill, the free ionic form of copper is the most important causal agent in determining acute lethality (*11, 13*). Complexation with bicarbonate reduces the bioavailability of copper to the gill, thereby reducing toxicity. By contrast, the inhibitory effects of copper on salmon olfactory neurons were only modestly affected by increasing bicarbonate and pH, even though free copper was dramatically reduced in these AFW formulations (Figure S3). This suggests that the ligand(s) in the salmon olfactory epithelium may have a relatively higher afÞnity for copper, and that bicarbonate-complexed copper is bioavailable to OSNs.

It is unlikely that copper inhibits olfaction by targeting the receptor proteins in the apical cilia or microvilli of OSNs that bind amino acids and other odorants. Previous studies in coho salmon (*26*), rainbow trout (*27*), and Atlantic salmon (*28*) have shown that amino acid–receptor binding is inhibited only at copper concentrations exceeding 100 μM, which is well above the concentration used in this study (20 μg/L, or 0.315 μM). Copper also does not appear to form nonstimulatory complexes with L-serine (*26*), an observation supported

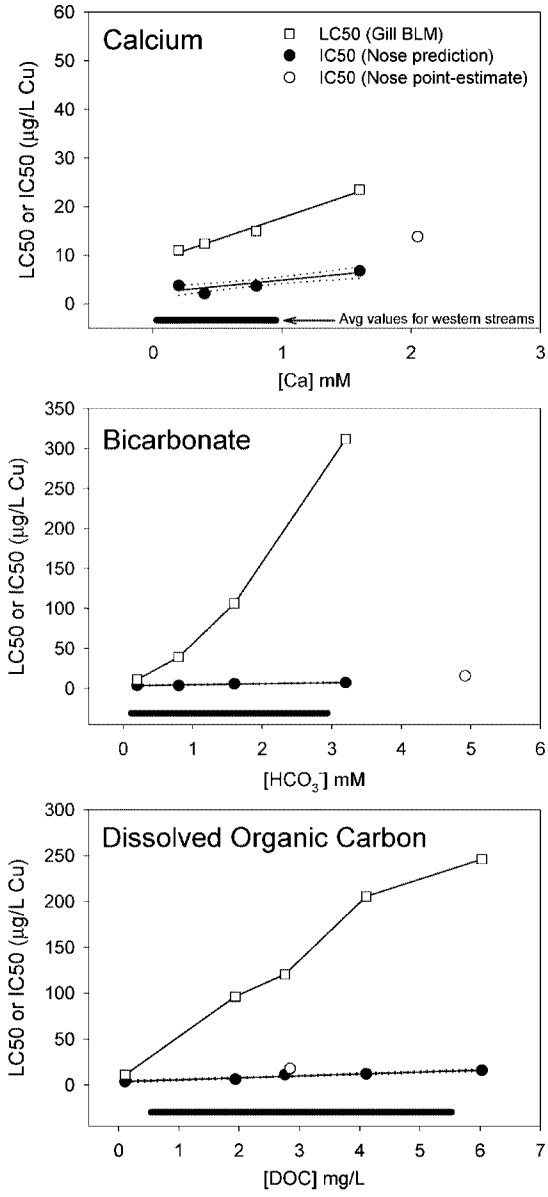

FIGURE 3. Water chemistry parameters are less protective at the fish nose than at the fish gill against toxicity from dissolved copper. Exposure concentrations of dissolved copper predicted to result in 50% toxic effect at the gill (open squares) LC50) or the nose (closed circles) IC50) for the various concentrations of calcium, bicarbonate, or DOC in artificial fresh waters tested. LC50s were generated by the Biotic Ligand Model (BLM) for each artificial fresh water treatment, as described in the text. IC50s were calculated from published dose–response relationships for copper in juvenile coho salmon, as described in the text. The open circle is a point estimate of the IC50 from the regression parameters (Table S1). The dark bars along the *x*-axes show the range of average measured concentrations of each parameter in western U.S. streams.

by the gradual decrease in EOG amplitude with copper exposure (not shown, but see Figure 4 in ref (*8*)), rather than an instantaneous drop in EOG amplitude. Signal transduction in OSNs involves a complex biochemical cascade (*16*). Mammalian studies have shown that micromolar concentrations of copper can block ion channels in neurons (*29–31*), and the metal may also interfere with transmembrane conductances in Þsh sensory neurons. In addition, if copper is translocated to the cytosol of salmon OSNs, it could potentially block the function of many key proteins, including, for example, voltage- and ligand-gated channels, G proteins, cyclases, kinases, phosphatases, and other proteins that control the excitable properties of sensory neurons (Figure S1; see also ref (*16*)). More work is needed to identify the biotic ligand(s) in the Þsh OSN.

Our present results contribute to a growing awareness that short-term exposures to environmental pollutants such as copper can interfere with the sensory biology of aquatic species (*32*). In addition to metals, recent studies have also shown that several current-use pesticides (e.g., refs *22*, *33*, *34*) as well as stream acidiÞcation (*35*) can cause chemosensory deprivation and altered olfactory-mediated behaviors in Þsh. The toxic effects of copper also extend to the lateral line (*36*, *37*), another important sensory system that underlies schooling and predator evasion behaviors in Þsh. In the case of PaciÞc salmon, a key priority for future research is to determine how a copper-induced loss of olfactory capacity affects life history traits that determine individual survival and lifetime reproductive success. These include, for example, olfactory imprinting, predation mortality, homing behavior, and spawning success. Information at these higher scales is needed to more effectively manage the conservation and recovery of ESA-listed salmon populations in the many western watersheds that are currently undergoing high rates of urban and suburban development.

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Supporting Information Available

Supplementary Methods are presented to give a more detailed description of copper speciation modeling and modeling of LC50s and IC50s. Supplementary Results and Discussion are included to describe copper speciation in artiÞcial fresh water (AFW) samples and to compare our current Þndings on the inßuence of hardness and alkalinity on copper olfactory toxicity to previously published results. Table S1 shows regression statistics for the inßuence of calcium, bicarbonate, and fulvic acid on copper olfactory toxicity. Figure S1 provides a schematic illustration contrasting the potential actions of copper at the gill epithelium and the olfactory epithelium. Figure S2 shows how copper-induced olfactory neurotoxicity (percent relative EOG amplitude) was measured. Figure S3 indicates how free ionic copper concentrations varied in AFW formulations based on direct measurements using the copper electrode and modeled estimates using VMINTEQ. Figure S4 compares our Þndings in this study for the inßuence of calcium and bicarbonate to previous studies of copper-induced olfactory toxicity in Þsh (*8*, *38*, *39*). This material is available free of charge via the Internet at http://pubs.acs.org.

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